

**The Claims Defining the Invention are as Follows**

1. A host-cell free method for culturing *Cryptosporidium* comprising the step of introducing *Cryptosporidium*, at a first lifecycle stage, into a host-cell free medium under conditions which enable the *Cryptosporidium* to progress to a second lifecycle stage.  
5
2. A method according to claim 1 wherein the first and second lifecycle stages are selected from the group consisting of: oocyst including excysted oocysts, sporozoite, trophozoite, meront I, merozoites (Type 1), meront II (early), meront II (late), merozoites (type II), macrogamont, microgamete and zygote.
- 10 3. A method according to claim 1 wherein the first lifecycle stage is an oocyst or a sporozoite and the second lifecycle stage is an oocyst, sporozoite or a trophozoite.
4. A method according to claim 1 wherein the second lifecycle stage is an oocyst.
5. A host-cell free method for culturing *Cryptosporidium* comprising the step of introducing *Cryptosporidium*, at a first lifecycle stage, into a host-cell free medium under conditions which enable the *Cryptosporidium* to complete its lifecycle.  
15
6. A host-cell free method for producing *Cryptosporidium* biomass from an initial inoculum of *Cryptosporidium* comprising the steps of: (i) putting the inoculum into a host cell free medium; and (ii) culturing the *Cryptosporidium* to increase the *Cryptosporidium* biomass.  
20
7. A method according to any one of the preceding claims wherein the host cell free medium is a buffered and balanced combination of inorganic salts, amino acids and vitamins.
- 25 8. A method according to claim 7 wherein the medium further comprises an additional constituent selected from the group consisting of: a carbohydrate source, antibiotics, bile and serum.

- 26 -

9. A method according to any one of the preceding claims wherein the medium has a pH at or about neutral pH.
10. A method according to any one of the preceding claims wherein the host cell free medium further comprises a second phase in the form of serum that has  
5 been treated to render it viscous or semi-solid.
11. A method according to claim 10 wherein the serum is coagulated.
12. A method according to claim 10 or 11 wherein the serum used to form the second phase is foetal calf serum.
13. A host-cell free method for culturing *Cryptosporidium* comprising the steps of:
- 10 a. isolating *Cryptosporidium* oocysts;  
b. excysting the isolated oocysts;  
c. resuspending the excysted oocysts in a host-cell free culture medium;  
d. incubating the culture prepared in step (c) under suitable conditions;  
and  
15 e. harvesting oocysts from the medium.
14. A method according to any one of the preceding claims wherein the *Cryptosporidium* belongs to the species selected from the group consisting of: *Cryptosporidium andersoni*, *Cryptosporidium parvum*, *Cryptosporidium muris*, *Cryptosporidium hominis*, *Cryptosporidium wrairi*, *Cryptosporidium felis*,  
20 *Cryptosporidium canis*, *Cryptosporidium baileyi*, *Cryptosporidium meleagridis*, *Cryptosporidium galli*, *Cryptosporidium serpentis*, *Cryptosporidium saurophilum* and *Cryptosporidium molnari*.
15. A host cell free medium capable of maintaining *Cryptosporidium* or enabling the progress of *Cryptosporidium* through its lifecycle, the medium comprising a  
25 buffered and balanced combination of inorganic salts, amino acids, vitamins and additional constituents.
16. A biphasic host cell free medium capable of maintaining *Cryptosporidium* or enabling the progress of *Cryptosporidium* through its lifecycle the medium

- 27 -

comprising a buffered and balanced combination of inorganic salts, amino acids, vitamins and additional constituents.

17. A medium according to claim 15 or 16 wherein the additional constituents are selected from the group consisting of: amino acid supplements, carbohydrate  
5 source, antibiotics, bile and serum.

18. A medium according to any of claims 15 to 17 with a pH about neutral.

19. A medium according to claim 16 wherein the second phase comprises serum that has been treated to render it viscous or semi-solid.

20. A medium according to claim 19 wherein the serum is foetal calf serum.

10 21. A method for preparing an immunogenic preparation comprising at least one *Cryptosporidium* antigen, the method comprising the steps of: (i) introducing *Cryptosporidium*, at a first lifecycle stage, into a host-cell free medium under conditions which enable the *Cryptosporidium* to progress to a second lifecycle stage; (ii) isolating the *Cryptosporidium* at the second lifecycle stage; and (iii)  
15 preparing a therapeutic preparation using the *Cryptosporidium* isolated from step (ii).

22. A method according to claim 21 wherein the second lifecycle stage is an extracellular lifecycle stage.

23. A method according to claim 21 wherein the second lifecycle stage is a trophozoite, merozoite or other extracellular gamont-like stage.  
20

24. A therapeutic composition comprising a therapeutically effective amount of *Cryptosporidium* cultured according to any one of claims 1 to 14 and a physiologically acceptable carrier.

25. A composition according to claim 24 comprising a whole cell extract of one or more *Cryptosporidium* lifecycle stages.  
25

26. A composition according to claim 25 comprising one or more *Cryptosporidium* lifecycle stages that have been treated to disrupt their cellular structure.

- 28 -

- 27.A composition according to claim 24 comprising at least one isolated and purified *Cryptosporidium* antigen.
- 28.A composition according to claim 26 wherein the cellular disruption has been achieved by a technique selected from the group consisting of: sonication,  
5 osmotic pressure, freezing, exposure to detergents such as sodium dodecyl sulfate (SDS), and heating.
- 29.A composition according to any one of claims 24 to 28 wherein the *Cryptosporidium* cells have been inactivated.
- 30.A method of preventing or treating a disease associated with *Cryptosporidium*  
10 infection in a subject comprising administering to the subject a therapeutically effective amount of a composition according to any one of claims 24 to 29.
- 31.A method for detecting *Cryptosporidium* in a sample comprising the steps of:  
(i) subjecting the sample to the culture method described herein; and (ii) detecting the *Cryptosporidium*.
- 15 32.A method for detecting *Cryptosporidium* in a sample comprising the steps of:  
(i) introducing the sample into a host-cell free medium under conditions which enable *Cryptosporidium* to progress to a further lifecycle stage; and (ii) detecting the *Cryptosporidium*.
- 20 33.A method for detecting *Cryptosporidium* in a sample comprising the steps of (i) introducing the sample into a host-cell free medium under conditions which enable the *Cryptosporidium* to complete its lifecycle; and (ii) detecting the *Cryptosporidium*.
- 34.A method according to any one of claims 31 to 33 wherein the sample is from a water source that is to be used by humans or animals.
- 25 35.A method according to claim 34 wherein the water source is a source of drinking water such as a dam, lake, river or rain catchment area.
- 36.A method according to any one of claims 31 to 35 wherein the *Cryptosporidium* is detected via visual examination.

- 29 -

37.A method according to claim 36 wherein the visual examination is via a microscope or some other means that enables any *Cryptosporidium* in the sample to be viewed.

5 38.A method according to any one of claims 31 to 35 wherein the *Cryptosporidium* is detected using PCR.

39.A method according to any one of claims 31 to 38 further comprising the step of pretreating the sample to concentrate any *Cryptosporidium* therein.

40.A method according to claim 39 wherein the pre-treatment comprises centrifugation of the sample.